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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the application of:

RAMEZ E. SHEHADA, ET AL.

Serial No.: 08/889,017

Filed: July 7, 1997

For: METHOD AND DEVICES FOR LASER INDUCED FLUORESCENCE ATTENUATION SPECTROSCOPY

Examiner: A. Israel

Group Art Unit: 2878

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Name

Signature Date

DECLARATION OF RAMEZ E. SHEHADA, PH.D.

Assistant Commissioner of Patents
Washington, D.C. 20231

I, RAMEZ E. SHEHADA, declare as follows:

1. I am a named inventor in the above-identified application.

2. I have a doctorate degree in Biomedical Engineering and have been actively conducting research in the fields of Biomedical Optics and Lasers for the past 9 years.

3. I have followed the prosecution of the above-identified application and have examined the Office Action of August 16, 1999 and the references cited in this application, including U.S. Patent Nos. 5,495,850 (Zuckerman) and 5,635,402 (Alfano et al.) and International Patent Application WO 97/08538 (Sevick-Muraca et al.).

4. In view of my study of Zuckerman, Alfano et al. and Sevick-Muraca et al., I set forth my observations in support of the Amendment submitted in response to the Office Action of August 16, 1999, to overcome the rejections under Sections 102 and 103.

[5] "The vector components $I_{\parallel}$ and $I_{\perp}$ are respectively and simultaneously detected by two optical detectors **88A** and **88B**, which can be photodiodes or photomultiplier tubes." **See [column 10, lines 45-48]**

Hence, the detectors **88A** and **88B** do not detect the fluorescence of the irradiated sample from a first and second distances from the sample, however, they detect the linearly polarized vector components $I_{\parallel}$ (parallel **86A**) and $I_{\perp}$ (perpendicular **86B**) of the fluorescence returned from a single location, which is the probe's tip **68**.

[6] "Wavelengths >400 nm pass through the dichroic mirror **62** and wavelengths from 400-420 nm pass through an emission filter **82** to a Wollaston prism polarizer **84**, which resolves the emitted fluorescence into its linearly polarized components parallel **86A** and perpendicular **86B** to the plane of excitation polarization." **See [column 10, lines 40-45]**

Upon reaching the apparatus, the returned fluorescence is resolved into its linearly polarized components parallel **86A** and perpendicular **86B** by the Wollaston prism polarizer **84**.

[7] "Since optics are reversible, the linearly polarized fluorescence emission from the probe tip **68** returns along the single mode polarization-preserving glass fiber and is collected by the objective lens **64**." **See [column 10, lines 37-40]**.

Hence, the emitted fluorescence is collected from the probe tip **68** by a single optical fiber **66**. Therefore, the fluorescence is not collected at first and second distances from the sample.

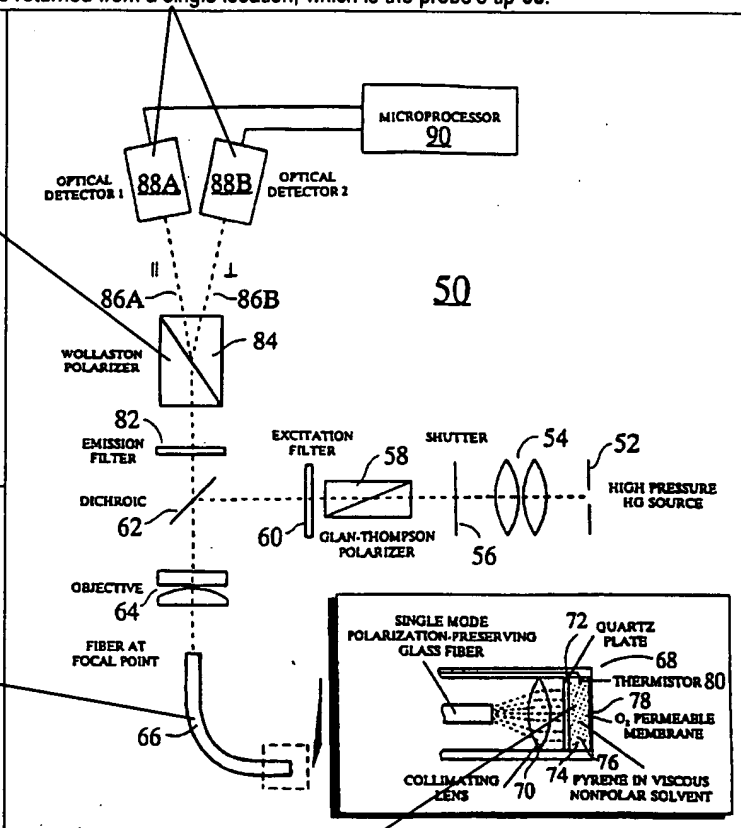

Figure 3

[8] "In the case of a narrow submillimeter catheter, the linearly polarized light passes directly to the O_2-sensitive tip **68**, whereas for larger diameter catheters the glass fiber **66** is mounted at the focal point of a collimating lens **70** which is used to provide uniform illumination of the catheter tip **68**." **See [column 10, lines 16-21]**.
"The catheter tip **68** contains a nonpolar viscous medium **74** such as paraffin or mineral oils in which pyrenebutyric acid or pyrene **76** is dissolved. Pyrene has a fluorescence lifetime approximately ten times that of pyrenebutyric acid or its salt form, thereby increasing the precision of the measurement, with an accuracy of PO_2 measurement of <1 mm Hg possible. The O_2-quenchable probe substance dissolved in a nonpolar viscous solvent is sealed within the catheter by an O_2 permeable membrane **78** such as polyethylene." **See [column 10, lines 24-34]**.

Hence, the polarized excitation radiation does not excite the sample itself, however, it excites a volume of an O_2-quenchable fluorescent probe substance **76** (i.e. pyrenebuteric acid or pyrene) encapsulated in the probe's tip and separated from the sample by an O_2 permeable membrane **78**. Therefore, the returned fluorescence is not measured from the sample at all, however, from single location within the tip **68** of the catheter.

Figure 3 of Patent number 5,494,850 by R. Zuckerman

[9] "The light passing through filters **55** and **57** is detected by photomultiplier tubes **59** and **61**, respectively, and converted into electrical signals, which are transmitted to electronics lock-in and then processed by computer **52**" **See [column 5, lines 64-67]**.

This indicates that the photomultiplier tubes **59** and **61** detect the fluorescence intensity returned through bundle legs **51** and **53** passing through filters **55** and **57**, respectively. Both fluorescence portions measured by the photomultipliers **59** and **61** were collected from a single spatial location facing the sample, which is which is the probe end **48** on the sample S.

[10] "The light emitted from slide S is collected by probe end **48** of bundle **47** and **then transmitted to** a second leg **51** and a third leg **53** of bundle **47**. Second leg **51** and third leg **53** of bundle **47** are equipped with filters **55** and 57, respectively, which function in the same manner as filters **17-1** and **17-2**, respectively, of system **11**." **See [column 5, lines 58-63]**.

Hence, the returned fluorescence is collected from a single spatial location facing the sample (i.e. the probe end **48**) and then split into two portions for the purpose of filtration with two different filters. There is no mention whatsoever of a first and second waveguides disposed at a first and second distances distance from the sample.

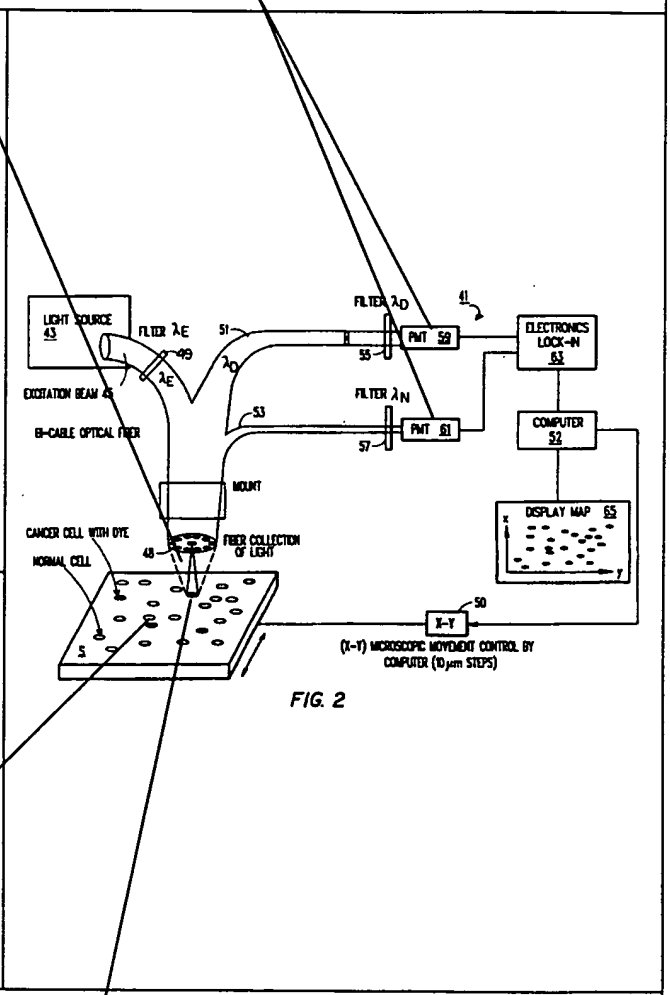

FIG. 2

[11] "X-Y movement of slide S to permit scanning of a larger area of the sample is effected by a motorized device **50**, which is controlled by a computer **52**." **See [column 5, lines 54-57]**.

Each scan of a given location is processed separately and independently from preceding and subsequent scans performed on neighboring locations. The X-Y movement is performed only to scan a larger area of the sample because the system can inspect a given location (i.e. whatever is facing the "probe end" **48**) at a given time.

[12] "The light transmitted from light source **43** is sent through a first leg **45** of a trifurcated fiber optic bundle **47**. Disposed within first leg **45** is a filter **49**, which is selective for light of a wavelength which will cause the dye in the sample to fluoresce, i.e. at a wavelength within the absorption curve for the dye." **See [column 5, lines 39-44]**. "The light emergent from the probe end **48** of bundle **47** illuminates a small portion of the slide S upon which the sample is smeared." See **[column 5, lines 53-55]**.

Figure 2 of Patent number 5,635,402 by R. Alfano et al.

[13] "Fluorescent light emitted in response to the excitation light is detected with sensor (**148**). The AC intensity and phase of the excitation and detected fluorescent light is provided to a processor (**160**) operatively coupled to sensor (**148**). Processor (**160**) employs the measured re-emission kinetics of excitation and fluorescent light to "map" the spatial variation of one or more fluorescence characteristics of the tissue (**100**)." **See Abstract [Lines 4-11]**

[14] "System **110** also includes detection subsystem **140** which has optic fibers **143** to detect photons emitted from tissue **100** from a number of corresponding detection sites." **See [page 7, lines 21-22]**.
"Sensors **128**, **148** and source **120** are operatively coupled to heterodyne subsystem **130**. Subsystem **130** is configured to obtain information about the phase, AC, and DC intensity of light detected with sensor **128** relative to light detected with the sensor **148** using conventional laser heterodyning techniques." **See [page 8, lines 1-4]**.
"In stage **216**, the phase, θ_{obs}, and log of AC intensity, M_{obs}, of the emission at each detection site "i" relative to the excitation light from source **120** are determined at the heterodyne (or offset) frequency". **See [page 9, lines 8-10]**.

Hence, the emission from each detection site is only processed relative to the source light detected by sensor **128**, however, separately and independently from the emissions of other detection sites. The only purpose for Sevick-Muraca to use multiple detection sites is to determine the spatial variation (or image) of the (1) quantum yield and the (2) lifetime of the fluorescence throughout the sample.

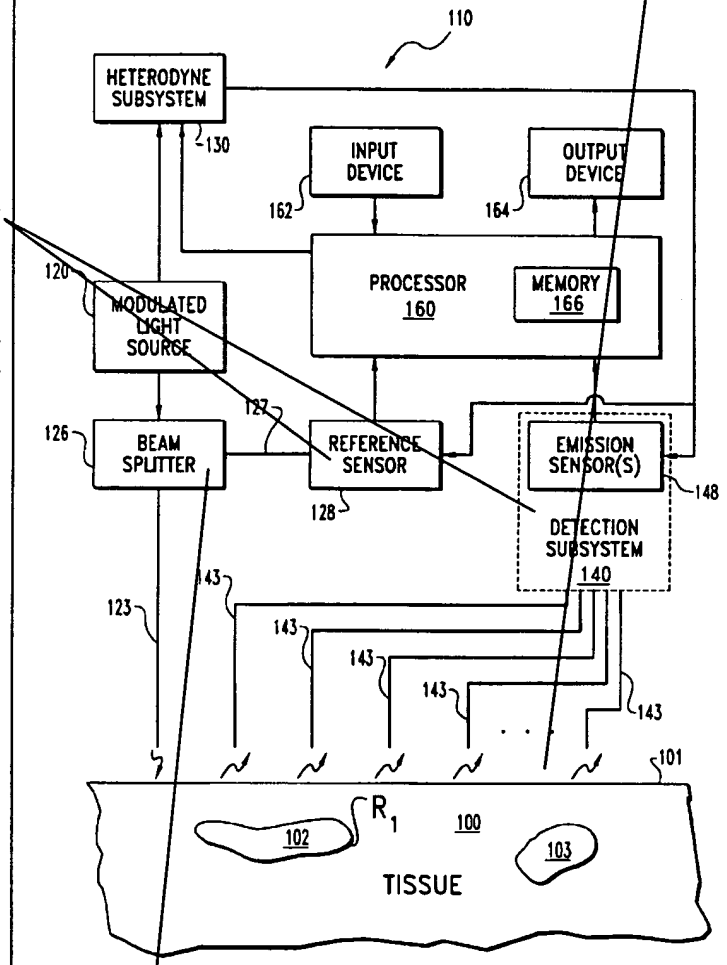

Fig. 1

[15] "Beam splitter **126** may be employed to direct a small portion of the excitation signal to reference sensor **128** for processing purposes." **See [page 7, lines 19-20]**.

Hence, the excitation light is detected by sensor **128** to be used as reference by the heterodyne subsystem.

Figure 1 of Patent number WO 97/08538 by Sevick-Muraca

16. The instant claimed invention, in my opinion, is patentable over the cited references. None of the cited references discloses or even remotely suggests a method or an apparatus using modulated fluorescence of a sample measured at two different locations from the sample, for comparison to each other to determine a modulation characteristic of the sample.

The undersigned, being hereby warned that willful false statements and the like so made are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any resulting patent issuing thereon, declares that all statements made on information and belief are believed to be true.

2/16/2000
Dated

Ramez Shehada
Ramez Shehada, Ph.D.